THE EFFECT OF LOW TEMPERATURE AND OF HYPOTONICITY ON THE MORPHOLOGY OF THE CLEAVAGE FURROW IN ARBACIA EGGS[1]

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When *Arbacia punctulata* eggs are exposed to low temperature during the first cleavage, a pronounced stalk develops between the daughter blastomeres. A stalk also develops at room temperature if the eggs are made to divide in hypotonic sea water or in sea water lacking calcium ion. The development of a conspicuous cleavage stalk is not a normal feature of the first cleavage in Arbacia, although it does occur regularly in some cells; for example, when fibroblasts divide. The object of the work reported here was to examine the conditions under which the stalk is formed in Arbacia and to relate these facts to current theories of the mechanism of cleavage. These particular experimental treatments were used because they were found to affect the appearance of the cleavage stalk.

Methods

Eggs of *Arbacia punctulata* in the first cleavage served as experimental material. Ovulation was induced by the removal of the oral half of the test; eggs emerging from the genital pores were collected in a dish of sea water. The eggs were allowed to settle and the sea water was decanted after which fresh sea water was added. Two such washings were carried out to minimize contamination by coelomic fluid. Fertilization was effected by the use of diluted "dry" sperm, and the sperm were never more than one hour old.

The fertilization membranes were removed by shaking. A heavy suspension of eggs was placed in a five-inch test tube, one-half full of the suspension, and shaken rapidly thirty times. Eggs so treated cleave in time with the controls. The best time for treatment is at 2½ minutes after fertilization, for if shaken earlier, many exovates are formed, and if shaken later, many eggs retain the fertilization membrane. The alternative method of removing the fertilization membrane by treatment with the hatching enzyme (Ishida, 1936) was not attempted.

The hyaline layer was removed in a few experiments by washing the eggs in calcium-free artificial sea water. This was accomplished by several decantations and additions of the calcium-free mixture. It was found that the hyaline layer regenerates somewhat if the eggs are returned to a solution possessing calcium ions; hence if eggs are to lack the hyaline layer, they must be allowed to cleave in the calcium-free mixture.

This study was largely accomplished by photographic means. Photomicro-

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graphs taken at intervals with Leica-Ibso apparatus, were projected as negatives (1,000 ×) and measurements made with dividers.

Temperature was maintained by means of a thermostatically controlled, water jacketed, glass well, mounted on the microscope stage and connected through a centrifugal pump to a water bath. By this means temperature could be maintained within ± 0.2° C. at or about 20° and within ± 0.4° C. at or about 10° C.

Artificial sea water lacking calcium ions was compounded according to the method of Shapiro (1941). This solution has an osmotic pressure and pH closely similar to that of normal sea water.

The hypotonic solutions were prepared either by the dilution of normal sea water or of the calcium-free mixture.

A few observations are presented on polyspermic eggs cleaving to three or to four cells in one division. Polyspermic development was induced by the method of Smith and Clowes (1924) which involves fertilization in pH 7.2 sea water and the return of the eggs to the normal pH of 8.4 within two or three minutes.

Results

Morphology of the cleavage furrow

The shape of the deepening furrow is markedly different under different conditions; it is influenced by temperature, concentration of calcium ion, tonicity and by presence or absence of the fertilization membrane.

Temperature

At temperatures between 20° C. and 30° C. there is normally no stalk in cleaving eggs whose fertilization membrane has been removed (free cleavage). The furrow is peaked at the apex (Figs. 1 and 2). At low temperatures, 6° to 12° C., a real stalk is formed during the latter part of the furrowing. This occurs whether the egg is enclosed in the fertilization membrane or not. At these low temperatures eggs undergoing membrane-free cleavage, come to resemble a dumbbell with a handle (Figs. 3 and 4).

Calcium ion or urea

Chambers (1938) described the short cleavage stalk which develops when *Arbacia punctulata* eggs divide in calcium-free solutions at room temperature. He used isotonic mixtures of sodium chloride and potassium chloride. In the present study also a short stalk occurred when the eggs were exposed to calcium-free sea water. Similarly a short stalk was figured by Moore (1930a and b) and by Motomura (1934), after treatment with urea solutions.

Fertilization membrane

It is a common practice to remove the fertilization membrane either by dissolving it in urea solutions or by shaking an egg suspension rather violently. These techniques allow the mitotic axis to become much longer and the furrowing is thus more readily followed. If the eggs are confined in the fertilization membrane at 10° C., the blastomeres tend to stay apart and the walls of the furrow are almost vertical (Figs. 11 and 12). At the end of the cleavage a stalk connects the two blastomeres. If this same experiment is varied so that the eggs divide within their

fertilization membranes at 10° C. and in calcium-free sea water, a cleavage stalk likewise develops. In this case, however, the stalk moves eccentrically until it is close to the fertilization membrane (Figs. 30 through 34). The difference is presumably due to the fact that the hyaline layer is dissolved in solutions lacking calcium ion and when the hyaline layer is missing the egg is able to slide around inside the fertilization membrane.

Membrane-free cleavage in polyspermic eggs

Polyspermic eggs may undergo free cleavage to form four cells in the first division. In such cases they frequently divide so that a symmetrical figure is seen from above. In this circumstance the four blastomeres each rest upon the bottom of the glass container (Figs. 15, 16, 17, and 18). Frequently one blastomere rests upon the other three at the end of the cleavage (Fig. 21). The former, more symmetrical type of cleavage is more readily followed. When such an egg begins to cleave it first flattens like a biscuit; at this stage it resembles a balloon around which two rubber bands have been placed at right angles. Such a balloon is flattened on the two surfaces where the rubber bands cross. Perhaps the egg, like the balloon, is subject to greater elastic tension in the region where the incipient furrows cross, and therefore flattens on these surfaces.

As seen from above, the egg periphery is roughly square, with corners rounded (Fig. 15); the wide furrows (at 10° C.) gradually sink towards the center with the apices of the furrows approaching one another. The upper and lower surfaces meanwhile remain relatively flat although the two flat surfaces slowly come together. The whole figure at the stage illustrated in Figure 16 resembles a balloon stretched closely over four tennis balls with two rubber bands placed at right angles. Finally a definitive stalk is formed (Fig. 18).

When the furrows first appear, the egg is to be considered as having two equatorial furrows; that is, two constricting rings (Fig. 29*a*), which cross each other. The quasi-independence of the furrows is demonstrated by some eggs which cleave in a similar way but *in which the furrows incise at different rates* (Figs. 19 and 20). In Figure 29*b* the furrow separating *ab* from *cd* is well in advance of the furrow separating *ad* from *bc*. This type of cleavage leads to a figure like Figure 20.

It appears that this curious type of cleavage is brought about by the development of two new ring-like tensions which develop around the necks of the individual blastomeres after the deeper furrow is well established. As a result of the deep primary furrow, four new isthmuses are established about the necks of the four incipient blastomeres (*cf.*, Fig. 29*b*). Perhaps the most significant feature of

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PLATE I

FIGURES 1 AND 2. Egg cleaving at 20° C. in sea water, fertilization membrane removed.
FIGURES 3 AND 4. Egg cleaving at 10° C. in sea water, fertilization membrane removed.
FIGURES 5 AND 6. Egg cleaving at 20° C. in 65 per cent sea water, fertilization membrane removed.
FIGURE 7. Egg cleaving within the fertilization membrane at 20° C. in sea water.
FIGURE 8. Late cleavage at 20° C. in 65 per cent sea water, fertilization membrane removed.
FIGURES 9 AND 10. Polyspermic egg fertilized in sea water at pH 7.2, transferred to normal sea water at room temperature until cleavage began. Cleaving at 10° C. in sea water.
FIGURES 11 AND 12. Eggs cleaving within fertilization membrane at 10° C.

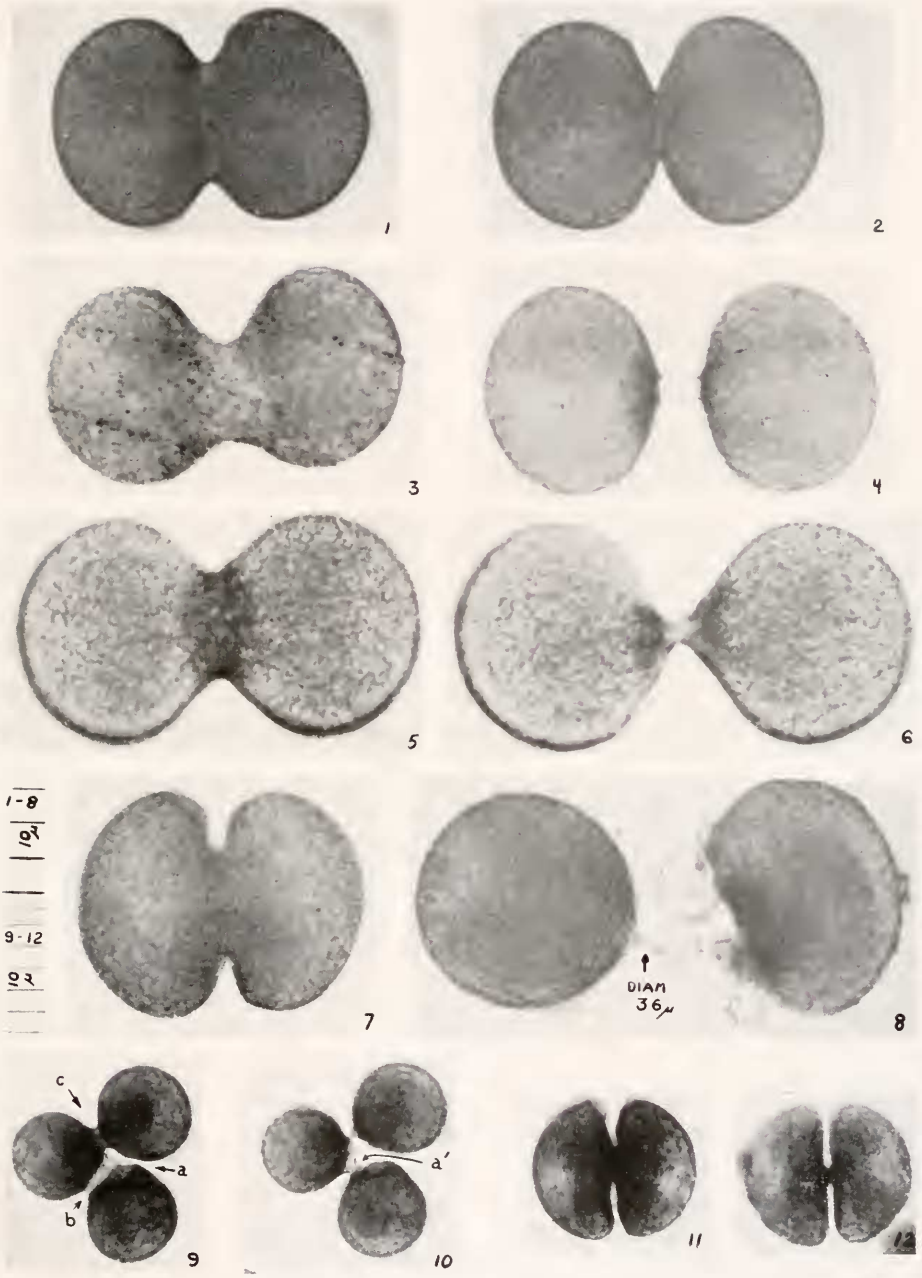


PLATE I

this type of cleavage is the bridge-like stalk which results (Figs. 23 and 24). In these latter figures note that one circumferential furrow deepened symmetrically and more rapidly than the other. The furrow which started later is very asymmetrical, being much deeper on one side (*cf.*, at the arrow) than the other. Eggs cleaving to three cells show a similar behavior (Fig. 22) and when cleavage is com-

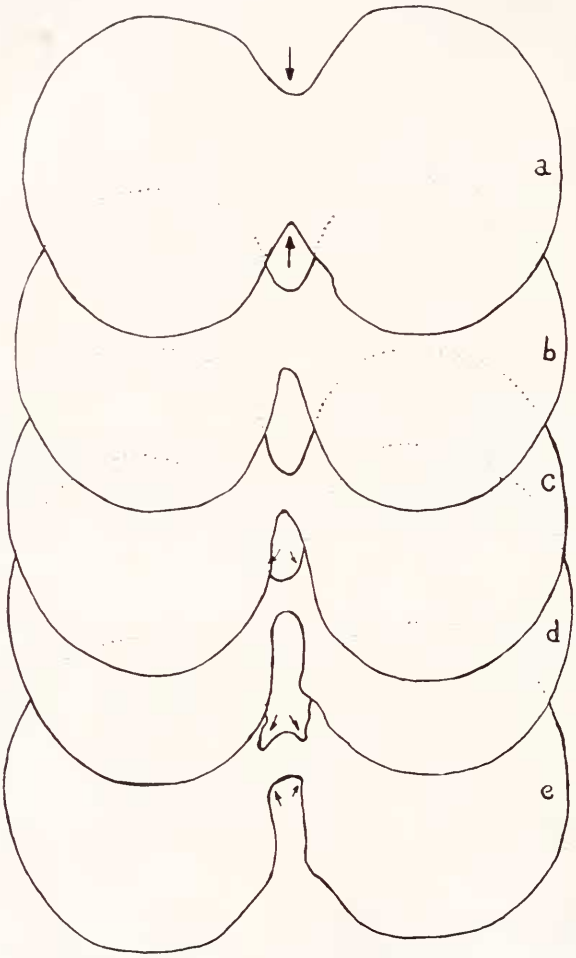


FIGURE 13. Series showing late cleavage and development of the cleavage stalk at 10° C. in sea water.

plete they may have a Y-shaped stalk, or if one furrow deepens more rapidly than the others, two stalks may connect to one blastomere (Figs. 9 and 10).

The speed of furrowing in polyspermic eggs cleaving to four cells may be as rapid as when two cells are being formed, yet it should be remembered that the amount of new surface formed is much greater when a sphere divides into four equal smaller spheres. The surface of a sphere divided into two spheres increases 26 per cent.

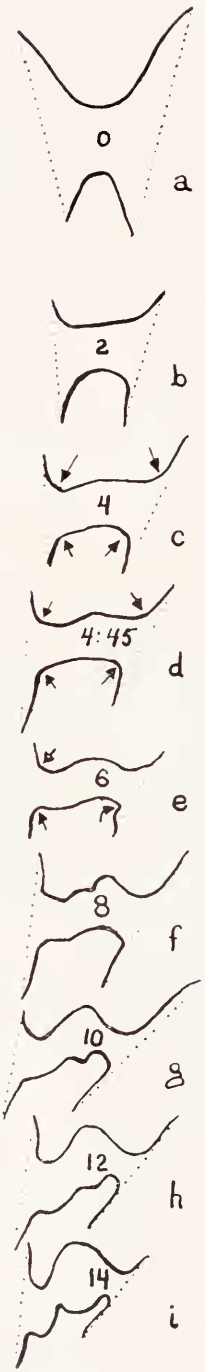


FIGURE 14. Series showing late cleavage and continued activity of cleavage stalk, during fourteen minutes in calcium-free sea water at 11° C.

if divided to four spheres the surface increases 58 per cent. A polyspermic egg cleaving to four cells forms about 26 per cent more surface than the normal first cleavage but it may do so in the same amount of time.

Membrane-free cleavage in hypotonic sea water and in hypotonic calcium-free sea water

Dilution of the sea water causes a swelling of the egg; it also causes an unusually wide furrow to develop during the cleavage and leads to the formation of a stalk at the end (Figs. 5, 6, and 8). This effect occurs at room temperature (20° C.). The stalk may become very long if the sea water has been diluted sufficiently. In mixtures of 65 parts sea water and 35 parts distilled water, for example, the stalk may finally be 30 micra long. This effect occurs either in the presence or absence of calcium ion. The stalk region is certainly a relatively rigid gel, for it has sufficient rigidity to push the daughter blastomeres far apart. Figure 8 and Figures 40 through 42 show the process of elongation in these extreme cases. Enlarged photographs of the stalk at these stages are shown in Figures 43, 44, and 45 with dimensions noted. In Figure 43 the stalk is only 4.4 micra in diameter at one point. In Figure 44 its minimum width is about 2 micra and it is over 22 micra long. In Figure 45 the constriction is completed. The stalk is still 5 micra wide at some points, but it is less than 3 micra in diameter for a third of its length. Chambers (*ibid.*) relates that a spherical oil drop lying within the egg in the furrow region is not deformed until the "external surface of the advancing furrow is 4 to 5 μ from the surface of the oil." If the egg pictured in Figure 43 has a cortex comparable in thickness, then the stalk must certainly be all gel by the time its diameter is reduced to 7 or 8 μ. One blastomere sometimes ruptures when eggs cleave in 65 per cent sea water. No endoplasm escapes if the stalk has closed. One such closed stalk is shown in Figure 28; it is 5 micra in diameter. The conclusion that the stalk is all gel (and yet it continues to constrict) is a most important conclusion for it strongly supports the contraction theory of cleavage of W. H. Lewis. Close inspection at high magnification fails to show any movement of granules located in the stalk. The active constriction of a 5 μ stalk is recorded in Figures 26 and 27.

PLATE II

FIGURES 15, 16, 17, AND 18. Cleavage of a dispermic egg, cleaving in calcium-free sea water at 10° C. Egg fertilized in pH 7.2 sea water, transferred to sea water at room temperature until beginning of cleavage. Time after fertilization: Figure 15, 72 min.; Figure 16, 74 min.; Figure 17, 88 min.; Figure 18, 190 min.

FIGURES 19 AND 20. Egg showing dispermic cleavage. Treatment as in Figures 15–18. Time after fertilization: Figure 19, 86 min.; Figure 20, 88 min.

FIGURE 21. Dispermic egg. Treatment as in Figures 15–18. One blastomere out of the horizontal plane.

FIGURE 22. Diagram illustrating two types of cleavage to three cells.

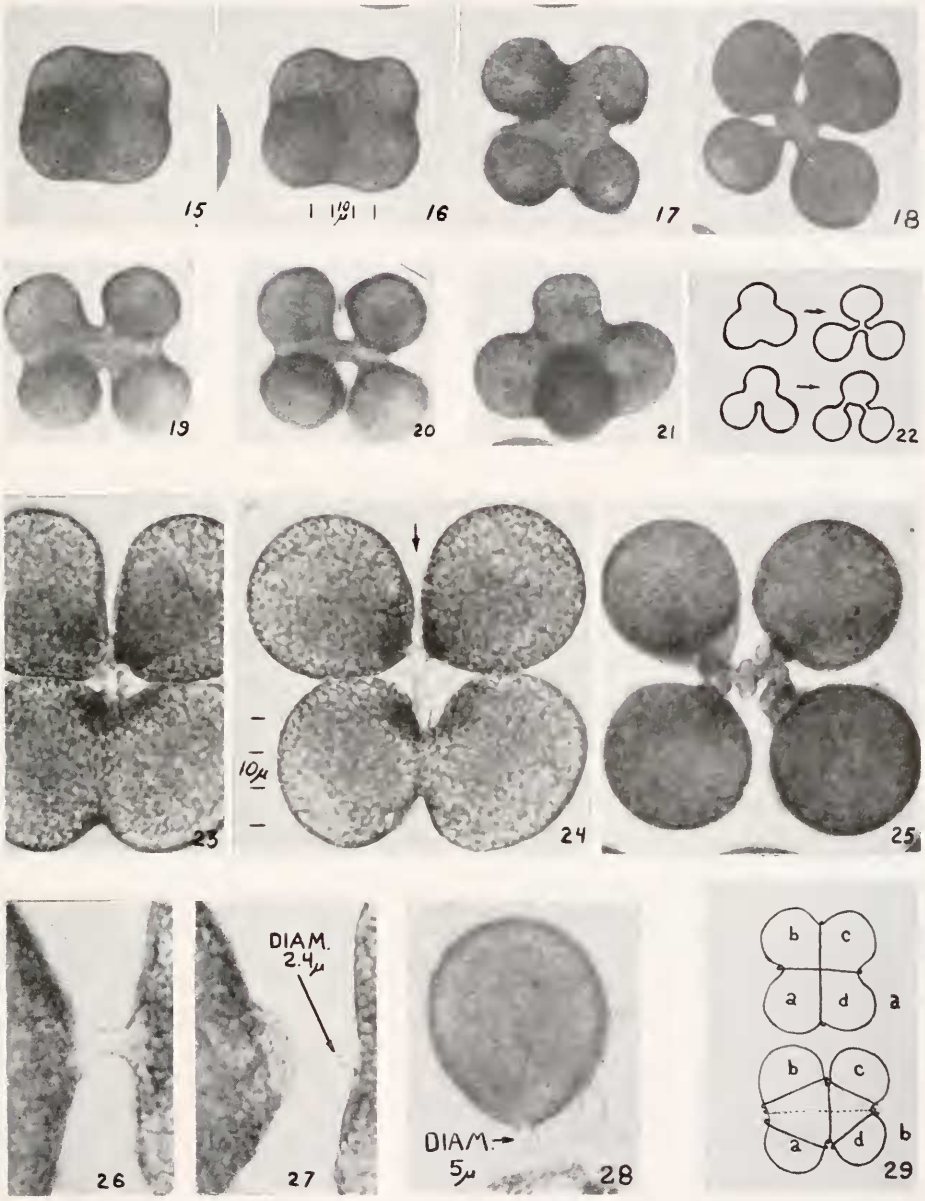
FIGURES 23 AND 24. Egg in 70 per cent sea water at 25° C., after accidental polyspermy. Time after fertilization: Figure 23, 50 min.; Figure 24, 52 min.

FIGURE 25. Dispermic egg cleaving in sea water at 11° C., following fertilization in pH 7.2 sea water.

FIGURES 26 AND 27. Late cleavage of egg in 65 per cent sea water. Room temperature. Time after fertilization: Figure 26, 83 min.; Figure 27, 85 min.

FIGURE 28. Closed stalk following rupture of one blastomere; 65 per cent calcium-free sea water.

FIGURE 29. See text.


15
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28
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a
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29

PLATE II

The stalk

The mitotic axis (greatest length) of eggs undergoing free cleavage becomes progressively longer at 10° than at 20° C. (compare Figs. 1 and 2 with 3 and 4); moreover the early furrow at 10° C. is much more blunt in contour than is the furrow of eggs at higher temperatures. A study of the final phase of cleavage under high power (Fig. 13) shows how the wide furrow is transformed into a stalk.

In Figure 13*a* the deepening furrow is still blunt with a diameter of about 14 micra. In Figure 13*b*, however, the stalk is beginning to square off. The arrows (Figs. 13*d* and *e*) indicate the region where the constriction is most active. The details are similar and are very clear in eggs cleaving in calcium-free sea water at 10° C. The series of diagrams shown in Figure 14, *a* to *i*, again show that the broad furrow first deepens until the diameter of the waist is about 7 or 8 micra (*a* and *b*), then the stalk is elongated by the constriction of the subequatorial cortex (*c* and *d*, see arrows); meanwhile the entire stalk is diminishing in diameter. The minimum diameter of the stalk is about 4 micra at 10° C. and in calcium-free sea water; in hypotonic solutions the diameter is often less. When the diameter of the stalk diminishes below 4 micra, it does so in local areas only (*cf.*, Fig. 14*g*, *h*, *i*).

Amoeboid activity and cleavage activity

Many workers have noted that the polar surface of the cell bubbles actively during cytokinesis (Bowen, 1920—in *Euchistus spermatocytes;* and Lewis 1942—in tissue culture fibroblasts). This is not the case with the egg of the sea urchin during the first cleavage, instead the polar surface remains smooth and inactive. However, a variety of agents, will cause the formation of blebs in the sub-furrow region. One such agent is hypotonic calcium-free sea water. The blebs usually begin to form after the completion of the major furrowing and they give rise to sizable spherules which are cut off by a process very much like cleavage (Figs. 46*a*, *b*, *c*). The inactivity of the polar surface may indicate that the cortex there is different from the equatorial cortex in Arbacia.[2]

Eggs that have been in the hypotonic medium for some time may show a sudden rush of endoplasm from one blastomere to the other, often causing the blastomeres to become very unequal in size (Fig. 47 and Figs. 35 to 39).[3] This endoplasmic flow is a very rapid one, usually lasting only two or three seconds. It is remarkable, however, that the flow is accompanied by a rapid deepening of the furrow, appearing as though a tension has been suddenly overcome, allowing the furrow to constrict much more rapidly than usual. The sub-cortical flow in such eggs may be down one side of the furrow, through the constricted stalk and up the other side of the furrow, yet the furrowing continues normally to completion (Fig. 47).

[2] The view that there is a special substance (a special type of plasmagel) located around the equator has been espoused by a number of workers, Marsland (1942) and Lewis (1942) among others. Beams and King (1937) are of the opinion that they have removed the "surface active material" of Ascaris eggs by centrifugation at 150,000 × gravity.

[3] The rush of endoplasm described is in this case related to cleavage. It resembles the amoeboid activity described by Moser (1940) after urea treatment. Moser, p. 77, cites other cases from the literature.

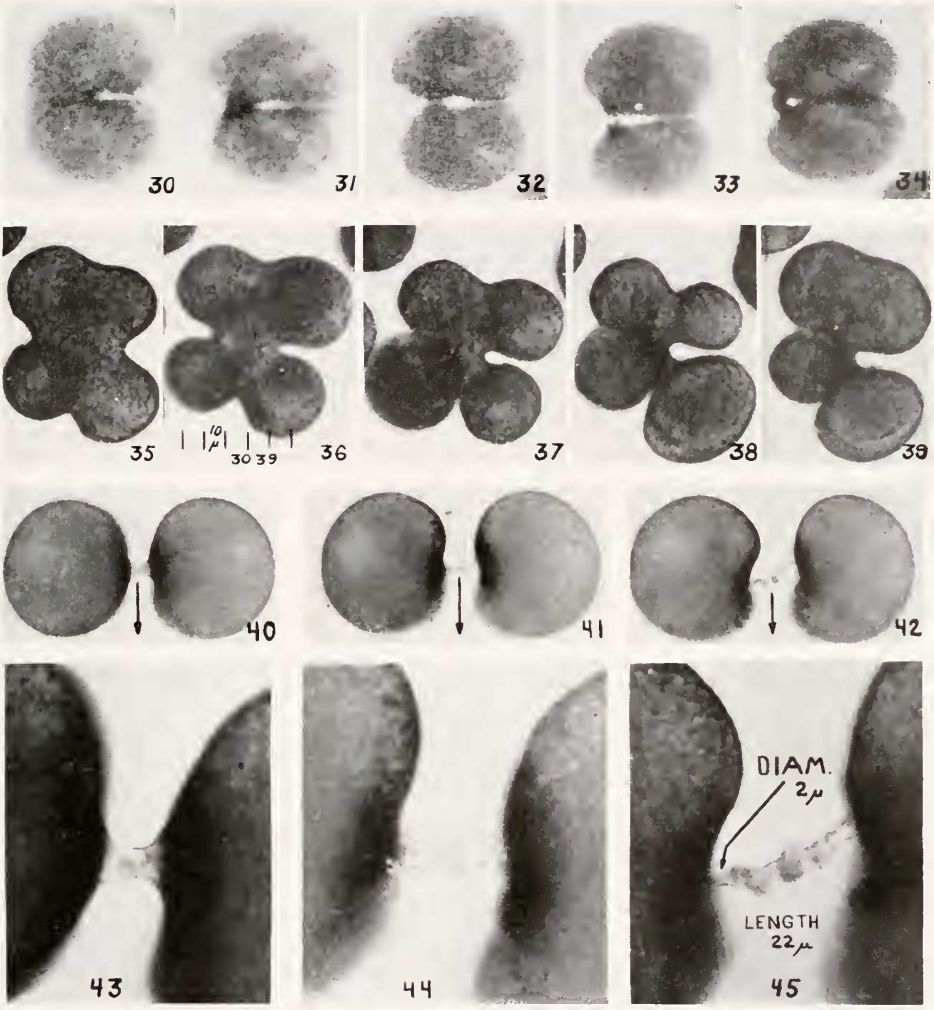

PLATE III

FIGURES 30, 31, 32, 33, AND 34. Eccentrically placed cleavage stalk. Eggs in fertilization membrane at 10° C., in calcium-free sea water.

FIGURES 35, 36, 37, 38, AND 39. Volume changes of individual blastomeres. Calcium-free sea water.

FIGURES 40, 41, AND 42. Elongation of the cleavage stalk; in 65 per cent sea water at room temperature. Time intervals: Figures 40–41, 1 min. and 40 sec.; Figures 41–42, 1 min. and 35 sec.

FIGURES 43, 44, AND 45. Enlargements of Figures 40, 41, and 42. The edges of the nearly transparent stalk have been retouched in Figures 4, 8, 26, 27, 28, 43, 44, and 45.

Discussion

The stalk

The occurrence of a stalk during the cleavage of the Arbacia egg is correlated with the degree of gelation of the furrow cortex. Both the observations made in this paper and those of other workers who have concerned themselves with the degree of gelation of the egg cortex confirm this. The results of several workers are summarized below:

Brown (1934): Cortical pigment granules are especially resistant to displacement by centrifugation during the division phase.

Chambers (1938): The furrow cortex resists disintegration after the two incipient blastomeres have been punctured at the poles.

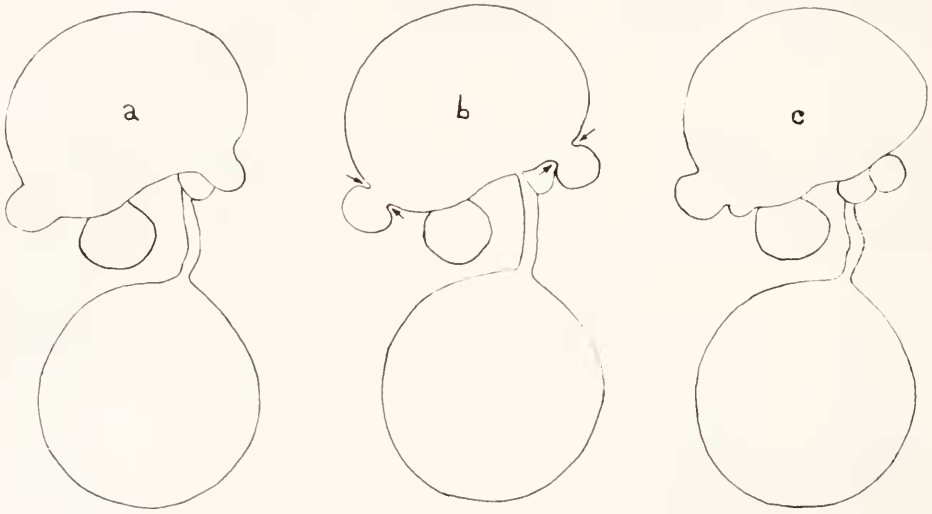

Figure 46

Brown and Marsland (1936): There is a quantitative decrease in the gel value of dividing eggs as the hydrostatic pressure is increased. Under high pressures the furrow regresses.

No one has yet recorded the effect of temperature, hypotonicity and lack of calcium ion upon the cortex of the dividing Arbacia egg, although these observations have been made upon the unfertilized egg. A brief summary of this work follows:

Costello (1938): It takes progressively longer to fragment the eggs as the temperature is lowered.

Cole (1932) and Harvey (1943): It takes longer to fragment eggs in hypotonic than in isotonic solutions.

Harvey (1945): Arbacia eggs break less readily in solutions possessing calcium ions than in solutions lacking calcium ions.

These treatments (low temperature, hypotonicity and calcium ion) are precisely the ones which favor the development of a cleavage stalk. It is possible that

these treatments may increase the elastic strength of the egg surface by toughening the extra cortical structures, but it is probable that they favor cortical gelation as well.

Hypotheses concerning the mechanism of cleavage; surface tension

Chambers and Kopac (1937) found that clean oil drops of the proper interfacial tension with sea water, will coalesce spontaneously with a naked egg (Arbacia, Lytechinus, and Echinometra). They state: "The tendency to coalescence in the furrow and polar zones of cleaving eggs (late amphiaster and later) was investigated and no difference was found." They used oils whose approximate tensions in contact with sea water were 30, 10, and 3 dynes per cm. The fact that coalescence occurs at all indicates a fluid layer around the egg periphery. Spontaneous coalescence does not occur in *Amoeba proteus* (Kopac and Chambers, 1937), which indicates a non-fluid surface. In view of these observations any surface tension hypothesis is untenable.

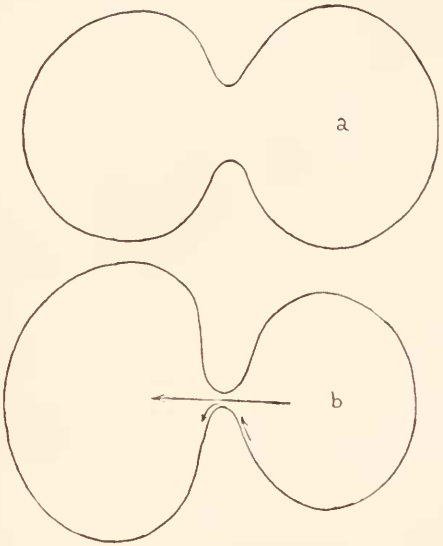

FIGURE 47

Subcortical currents

Chambers (1938) has hypothesized that cleavage results from the activity of "the sub-cortical currents (which) sweep around the two asters and add gelating material to the inwardly growing cortex." In this hypothesis he combines his own observations with those of Schechtman (1937) on localized cortical growth during the cleavage of the amphibian egg. It was shown in the present paper (page 280) that normal furrowing may be associated with abnormal currents, which argues against the importance of such currents for division; moreover Lewis (1942) found no currents in the dividing fibroblast.

Astral cleavage

An astral theory of cleavage, much modified from Gray (1931), has been elaborated by Katsuma Dan (1943). He believes that the asters are composed of radiate fibers with intrinsic rigidity; he considers them to be anchored to the cortex; he believes that the rays cross at the equator; and he believes that the spindle elongates autonomously. The following quotation (Dan 1943) summarizes his theory of cytokinesis: ". . . it was also shown that this concept of the mechanism of cell division is adequate to explain the stretching phase of the furrow surface. That is, when two such radiate asters are pushed apart, they can in turn, push the cell membrane of the polar region somewhat as a paint brush would push some object. As they travel away, however, since they enclose the fluid endoplasm within the interspaces of their rays, the fluid endoplasm is carried away from the equatorial region and the cortex there is sucked in, giving rise to a furrow. The cortex is stretched as it is pulled in by the suction."

The strength of Dan's hypothesis lies in its ability to explain the differential stretching and shrinkage of the surface which he and his coworkers observed (Dan, Yanagita, and Sugiyama, 1937; Dan and Yanagita, 1938; Dan, 1943) and for which no other explanation has been forthcoming. It appears that Dan's hypothesis will explain such unusual cleavages as are pictured in Figures 9 and 10 of the present paper. It could be assumed that one element of the tripolar spindle elongated before the others causing the asters to move apart, and by the suction mechanism, causing the development of the initial furrow (Fig. 9 at *a*). On this assumption the development of the other furrows (Figs. 9*b* and *c*) begins later, presumably because the other two spindles begin their elongation later. The secondary furrow (Fig. 10 at *a'*) is presumably caused by the suction resulting from the separation of the lower two asters. Similar explanations would doubtless serve for the tetra-astral cleavages shown in Figures 23 and 24 of the present study. One can imagine also that the crossing rays from all four asters, if they became attached to the cortex, would explain the flattening of the upper and lower surfaces of the egg observed in Figure 15.

Dan's hypothesis is not in accord with the observations presented here concerning the continued elongation of the cleavage stalk in hypotonic sea water for it is impossible to see how the astral suction mechanism could explain the further constriction of a long, completely gelated stalk.

The main weakness of the astral suction hypothesis lies in its limited scope. It fails to explain undoubted cases of anastral cleavage (tissue culture, for example) frequently noted in the literature. Dan's easy conclusion that all of these anastral cases are explainable by his astral suction hypothesis (". . . it is possible to imagine that in cells of the anastral type, similar gelation systems may be existing although they cannot be discerned morphologically") is unconvincing.

One of Wilson's observations is discordant with Dan's hypothesis. Wilson observed, in a form which normally has asters, that a spindle need not be present for complete cleavage to occur. He found that a cleavage furrow may cut in around the base of an isolated aster and result in a complete cleavage. Compare Wilson (1901), page 376 and Figure 11.

In one of Chamber's microdissection experiments he bisected the partially cleaved egg in a plane at 45° to the plane of the furrow (1924, Fig. 36). The cut resulted

immediately in two cells. However, the original furrow remained on each artificially produced blastomere and, on each, the furrow gradually cut through forming two small "cells" as well as two large ones. This continued cleavage seems to be quite unexplainable by Dan's hypothesis which requires crossed astral rays, an elongating spindle and a suction produced by the separation of the asters.

Cortical growth or cortical contraction?

Schechtman has proposed another theory of the mechanism of cytokinesis. He suggested (1937) that the furrow cortex grows by the "intussusception of clear cytoplasm," but simple growth of the equatorial cortex would not be expected to cut the egg in half. Other factors must account for the inwardly directed furrow and its narrowing. It seems clear that there is a stretching of the egg cortex at the time of furrowing as concluded by Dan et al. (1937, 1938), by Schechtman (1937) and by Motomura (1940), but whether the stretching is active (the result of growth) or whether it is passive and due rather to a contracting ring at the head of the furrow (Lewis, 1942), is not easy to decide. Schechtman is of the opinion that "Cleavage is initiated by a contraction of the egg cortex at the site of the future furrow." And he notes that the "Cortex becomes thicker and bulges toward the egg interior." He therefore uses both contraction and cortical growth in his complete hypothesis. The observations made in this paper on the continued constriction of small stalks after they consist entirely of gelated material are taken as strongly favoring the constricting ring theory of Lewis. For if the gelated stalk is able to contract at that late stage of cleavage, it seems reasonable to suppose that it possesses contractile power earlier. The direction of contraction is ringwise about the equator (Fig. 29*a*) and it is to be expected that such contraction would draw stained areas out into fine lines as Schechtman observed, if such areas are located in the furrow or subfurrow region.

It would be illuminating to know whether or not kaolin particles placed *around* the equator would be brought closer together during the furrowing but no one has made these observations.

Amoeboid activity and bleb formation

One can scarcely observe the amoeboid behavior of eggs in hypotonic media and particularly the "normal" false cleavages which occur during the amoeboid phase preceding pronuclear fusion in the nematode egg (Spek, 1918), without being convinced that a fundamental similarity exists between amoeboid motion and cleavage. Moreover the abscission of blebs is strikingly similar to cleavage.[4] It is suggested that any deforming force which establishes an isthmus about the cell or a part of the cell will result in the development of a contracting ring disposed around the isthmus, provided that the egg is in the cleavage phase. This view would explain why the normal egg, deformed by the elongating spindle, cleaves at the equator. It would explain why cleavage planes cut in around the base of cytasters which are unconnected to a spindle (Wilson, 1901) and it would explain why blebs formed in

[4] Very recently Holtfreuter (1946) has suggested "that in normal cytoplasmic division the activity of the nucleus and of the endoplasm are of a mere secondary importance." He observed that isolated, embryonic amphibian cells may develop annular constrictions which lead to the fragmentation of the cell. He considers, however, that the contraction occurs in the membrane rather than in the plasmagel layer.

the sub-furrow region may cut off from the remainder of the egg as reported above. This hypothesis also agrees with the idea that the enlarging gelated asters play a mechanical role in localizing the furrow.

Summary

1. Under certain conditions the eggs of *Arbacia punctulata* develop a cleavage stalk between the first two blastomeres. No stalk forms in sea water if the temperature is in the 20° C. to 30° C. range; low temperature (10° C.) causes the development of a stalk in sea water; a short stalk develops in isotonic calcium-free sea water at 20° C.; a very long stalk develops if eggs are cleaving in hypotonic sea water (65 per cent).

2. The effect of the above treatments on the appearance of cleaving dispermic eggs is described.

3. Evidence indicates that stalks of 8 micra diameter are all gel, yet in hypotonic sea water they continue to constrict and elongate. This is good evidence that the cortical gel has inherent contractile properties.

4. It is hypothesized that any event which deforms the Arbacia egg (if it is in the "cleavage phase") leads in some way to an orientation of contraction around the isthmus. The deforming force may be an enlarging aster, an elongating spindle, or an endoplasmic flow.

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